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Developmental Biology

# Ontogeny of the prohormone convertases PC1 and PC2 in the mouse hypophysis and their colocalization with corticotropin and α-melanotropin

**(mouse pituitary PC1 and PC2/*in situ* hybridization/immunocytochemistry/differential processing of proopiomelanocortin)**

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**ABSTRACT** **In the adult pituitary, anterior lobe corticotrophs and intermediate lobe melanotrophs differentially process proopiomelanocortin (POMC). Within the corticotrophs, POMC is processed mainly to corticotropin (ACTH) and β-lipotropin, while α-melanotropin (αMSH) and β-endorphin are the major end products in the melanotrophs. The observed transient presence of αMSH-like immunoreactivity during ontogeny suggested an age-dependent variation in POMC processing in the adenohypophysis. In this tissue, cell-specific POMC products are likely the result of differential expression of the two known prohormone convertases PC1 and PC2. In the present ontogeny study done in the mouse intermediate and anterior pituitary, we examined how the expression pattern of PC1 and PC2 mRNA transcripts correlates with that of ACTH and αMSH-like immunoreactivities. Our data demonstrated that both PC1 and PC2 transcripts can be detected in the presumptive adenohypophysis starting on embryonic day 15 (E15). In the intermediate lobe, PC1 and PC2 mRNAs appear on E18 and E16, respectively, and their levels increased during ontogeny, reaching maximal expression in the adult. Similarly, PC1 expression in the anterior pituitary increased from E15 to adulthood. However, PC2 mRNA expression peaked between postnatal days 1 (P1) and 14 (P14) and then decreased to adult levels. The distribution of PC1 and PC2 immunoreactivity is nicely correlated with the *in situ* hybridization data. In the anterior lobe, during the P1–P14 postnatal period, PC2 immunoreactivity was detected within cells synthesizing an αMSH-like peptide(s). This observation substantiates our earlier biochemical data suggesting that PC2 is the important convertase in the processing of POMC into αMSH. Furthermore, the demonstrated variation in the relative ratio of PC1/PC2 expression during ontogeny rationalizes the observed plasticity of POMC processing in the adenohypophysis. It is expected that β-endorphin processing will follow that of αMSH.**

PC1 (1–3) and PC2 (1, 4) are proprotein and prohormone converting enzymes responsible for cleavage of a number of precursors at distinct pairs of basic residues (5–8). In the pituitary, both PC1 and PC2 are expressed in the intermediate and anterior lobes (1, 2) and have been shown by *in situ* hybridization (ISH) to be colocalized with proopiomelanocortin (POMC) (9). The processing of POMC by PC1 and PC2 has been documented for the corticotropin (ACTH)/α-melanotropin (αMSH) and β-lipotropin (βLPH)/β-endorphin (6, 7) and the N-terminal glycopeptide segments of this precursor (10). Accordingly, PC1 would be responsible for production of ACTH and βLPH with some β-endorphin. PC2 would produce β-endorphin and either alone or together with PC1 would generate ACTH-(1–17). The transformation of the latter peptide into αMSH [*N*-acetyl-ACTH-(1–13)-amide] requires the action of carboxypeptidase E and the amidation and N-acetylating enzymes (5–7).

In the adult mouse pituitary, αMSH is produced in the melanotrophs (11) and only in a small number of atypical corticotrophs (12). This distribution resembles that of PC2 mRNA expression, which is elevated in the intermediate lobe melanotrophs and is low in the anterior lobe corticotrophs (2, 9). However, anterior lobe αMSH levels are more elevated during development than in the adult (13–16). These observations suggest that this differential POMC processing pattern may be dependent on the relative expression levels of the prohormone convertases. Accordingly, in the present study examining the ontogeny of PC1 and PC2 in the pituitary by a combination of ISH and immunocytochemistry (ICC), we established a relationship between the expression of these prohormone convertases and the levels of ACTH and αMSH. Finally, in the anterior pituitary, we also show a nice correlation between the transient production of αMSH and the expression level of PC2.

## MATERIALS AND METHODS

**Animals.** Six adult male mice CD1 (≈30 g) and 26 time-pregnant CD1 females (Charles River Breeding Laboratories) were used. The fetuses were grouped according to embryonic age—11, 12, 13, 14, 15, 16, 17, 18, and 20 intrauterine days of life (E11, E12, etc.); the neonates were obtained at postnatal days 1, 3, 5, 7, 9, 11, and 14 (P1, P3, etc.). For ISH, the animals were sacrificed by cervical dislocation. The pituitaries or the whole embryo was rapidly dissected out and cooled, embedded in OCT medium, frozen at −30°C in isopentane, and cut into 14-μm sagittal (embryo) or coronal (all others) sections. For ICC, we used paraffin tissue sections fixed by immersion (embryo) or transcardiac perfusion and immersion for 2 hr (all others) in cold 4% formaldehyde/0.34% L-lysine/0.55% sodium periodate in 0.05 M phosphate buffer as described (17).

**RNA Probes.** The antisense uridine 5′-[γ-[$^{35}$S]thio]triphosphate-labeled complementary RNA probes were generated by using T7 RNA polymerase for mPC1 (2) and T3 RNA polymerase for mPC2 (1). The cDNA sequences were inserted in Rc/CMV and Bluescript SK+ plasmids and were linearized with *Kpn* I to yield an mPC1 probe of 520 bp (specific activity, 114 Ci/mmol; 1 Ci = 37 GBq) or with *Sty* I resulting in an mPC2 probe of 506 bp (specific activity, 115

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Abbreviations: POMC, proopiomelanocortin; ACTH, corticotropin; βLPH, β-lipotropin; αMSH, α-melanotropin; ICC, immunocytochemistry; ISH, *in situ* hybridization; βLH, β subunit of luteinizing hormone; βFSH, β subunit of follicle-stimulating hormone; E15, embryonic day 15; P1, postnatal day 1.

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Ci/mmol), both containing the 3′ end of the cDNAs. The control sense RNA probes were generated from the same plasmids when T7 RNA polymerase was used.

**ISH.** ISH was carried out with cryostat tissue cuts as described (1, 2). All hybridization solutions were RNase-free. All tissue slides from different ages were hybridized in the same experiment and exposed 11 days for emulsion autoradiography. After ISH, the tissue sections were stained with cresyl violet and viewed under dark- and light-field illumination.

**Northern Blots.** Northern blot analysis was carried out as described by Day *et al.* (9), with $^{32}$P-labeled mPC1 and mPC2 RNA probes and 10 μg of total RNA obtained from different developmental stages of dissected pituitary neurointermediate and anterior lobes. Because of the small sizes of the embryonic neurointermediate lobe (E18–E20) the quantitative data could not be determined.

**Antibodies.** The rabbit polyclonal antibodies used (8) were AbC-mPC1 and AbC-mPC2, which are directed against the C-terminal segment 629–726 of mPC1 (2) or 529–637 of mPC2 (1), respectively. The specificity of these antibodies was demonstrated by their specific recognition of PC1 (87 or 80 kDa) or PC2 (75 and 65 kDa) and did not show any cross-reactivity between them (8) and by immunofluorescence on Sf9 recombinant insect cells expressing pro-PC1 or pro-PC2 (18). Control incubations with excess mPC1 or mPC2 antigens blocked the immunoreactivity. The other rabbit antibodies were the anti-ACTH (17); anti-αMSH, cross-reacting with both acetylated and desacetyl-αMSH, ACTH-(1–13)-$NH_2$, but not with ACTH-(1–13) or ACTH-(4–10) (19); anti-β subunit of luteinizing hormone (βLH) and anti-β subunit of follicle-stimulating hormone (βFSH) (A. F. Parlow, National Institutes of Health) (17).

**ICC.** The immunofluorescence staining–restaining method of Tramu *et al.* (20) was used, allowing sequential staining of two different antigens on the same section. The following primary antibody dilutions were used: mPC1 and αMSH, 1:150; mPC2, ACTH, and βFSH, 1:200; and βLH, 1:1500. The second antibody consisted of rhodamine-labeled goat anti-rabbit IgG (Cedarlane Labs) diluted 1:10. We found that the best ICC results were obtained with sections treated for 30–45 sec with a solution of 0.5% (wt/vol) potassium permanganate and 0.25% (vol/vol) sulfuric acid. Negative controls were done with antibodies blocked by preadsorption of AbC-mPC1 or AbC-mPC2 with the C-terminal antigens.

## RESULTS

**Developmental Gene Expression of PC1 and PC2 in Mouse Pituitary.** ISH results demonstrated that PC1 mRNA was detectable on E15 in the ventral aspect of the primordium of the anterior pituitary (Fig. 1 *A* and *A′*). While the intensity of the signal in this tissue varied little between E16 and E17 (Fig. 1 *B* and *C*), from E18 on it increased consistently until adulthood (Fig. 1 *D–F*). In the intermediate lobe, PC1 transcripts were visible after E18 (Fig. 1 *D–F*).

In the ventral bit of the Rathke's pouch, PC2 mRNA was clearly observed on E15 (Fig. 2 *A* and *A′*), but lower levels were detected between E16 and E20 (Fig. 2 *B–D*). Furthermore, during P1, P7, and P14, anterior lobe PC2 mRNA expression increased to maximal levels (Fig. 2 *E–G*), followed by a subsequent decrease until adulthood (Fig. 2*H*). In the intermediate lobe, PC2 mRNA was first detected at E16 (Fig. 2*B*). Thereafter, PC2 labeling increased continuously throughout both prenatal and postnatal periods until adulthood (Fig. 2 *B–H*). A semiquantitative analysis of these results is presented in Table 1.

**Northern Blot.** The developmental expression of PC1 and PC2 was also examined by Northern blot analysis in the neurointermediate (Fig. 3 *A* and *C*) and anterior (Fig. 3 *B* and *D*) mouse pituitaries obtained from embryos, neonates, and adults. In the neurointermediate lobe, PC1 and PC2 expression increased gradually from the first postnatal day to adulthood. The mRNA levels increased postnatally, reaching maximal levels at P42 (for PC1) and P28 (for PC2), with no

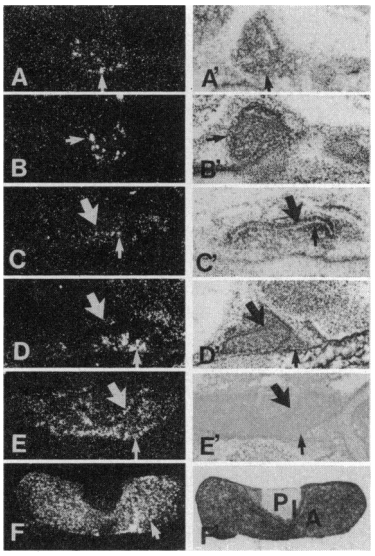


FIG. 1. ISH of PC1 in the pituitary during development. (*A–F*) ISH signals are seen as white spotted grains (thin arrows in pars distalis and heavy arrows in pars intermedia) over a dark background. (*A–F*) For comparison, the corresponding structures of the developing mouse pituitaries stained with cresyl violet are shown in light field. *In situ* data are shown for E15 (*A* and *A′*), E16 (*B* and *B′*), E17 (*C* and *C′*), and E18 (*D* and *D′*) and for P1 (*E* and *E′*) and adulthood (*F* and *F′*). A, anterior lobe; I, intermediate lobe; P, posterior lobe. (*A–D*, ×20; *E*, ×10; *F*, ×10.)

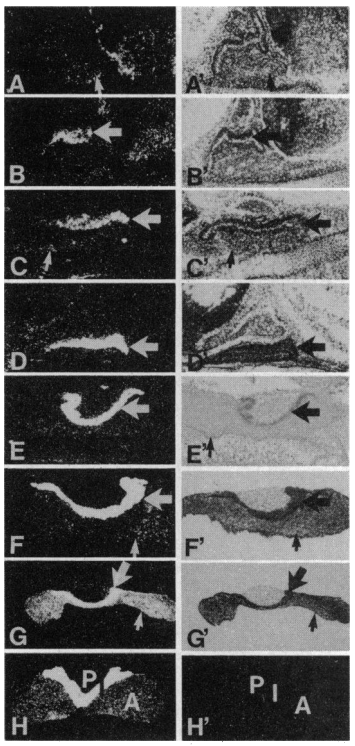


FIG. 2. ISH of PC2 in the pituitary during development. *In situ* data are shown for E15 (*A* and *A′*), E16 (*B* and *B′*), E17 (*C* and *C′*), and E18 (*D* and *D′*) and for P1 (*E* and *E′*), P7 (*F* and *F′*), and P14 (*G* and *G′*) and adulthood (*H* and *H′*). Abbreviations are as in Fig. 1. (*A–D*, ×20; *E*, ×10; *F* and *G*, ×10; *H*, ×10.)

Table 1. PC1 and PC2 mRNA transcripts and immunoreactivities in developing mouse pituitary gland

| | Age | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E11–E14 | E15 | E16 | E17 | E18 | E20 | P1 | P3 | P5 | P7 | P9 | P11 | P14 | AD |
| PC1 IL | | | | | | | | | | | | | | |
| ISH | − | − | − | − | ± | + | ++ | * | * | ++ | * | * | ++ | +++ |
| ICC | − | − | − | − | ± | + | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| PC1 AL | | | | | | | | | | | | | | |
| ISH | − | + | ++ | + | ++ | ++ | ++ | +++ | * | * | +++ | * | * | +++ |
| ICC | − | − | − | ± | + | + | ++ | ++ | +++ | +++ | +++ | +++ | ++++ | +++++ |
| PC2 IL | | | | | | | | | | | | | | |
| ISH | − | − | ++ | ++ | +++ | +++ | ++++ | * | * | ++++ | * | * | +++++ | +++++ |
| ICC | − | − | + | ++ | ++ | ++ | +++ | +++ | +++ | +++ | +++ | ++++ | ++++ | +++++ |
| PC2 AL | | | | | | | | | | | | | | |
| ISH | − | + | + | + | + | + | ++ | * | * | +++ | * | * | ++++ | ++ |
| ICC | − | − | − | ± | ± | − | − | + | ++ | ++ | +++ | ++++ | +++ | + |

Data were obtained by specific ISH and ICC. Immunoreactivities are on a scale from + to +++++; −, not detected; *, not determined. AD, adulthood; IL, intermediate lobe; AL, anterior lobe.

appreciable decline until adulthood. In the anterior pituitary, PC1 and PC2 expression was detectable on E18. PC1 expression increased almost continuously during development (Fig. 3*B*). In contrast, PC2 showed elevated levels of expression within the first 2 postnatal weeks (peaking between P1 and P14) and then declined after P14 to reach adult levels at P28 (Fig. 3*D*).

**ICC Localization of PC1.** A weak PC1 immunoreactivity was detected on E16 in both the intermediate and anterior lobes (Fig. 4*A*). In the intermediate lobe melanotrophs, low levels of PC1 immunoreactivity were detected at all developmental stages analyzed (Fig. 4 *A–D*). In agreement with the ISH data, PC1 immunoreactivity in the anterior lobe did not change appreciably during the prenatal period (Table 1), but it increased substantially from P1 to P3 through adulthood (Fig. 4 *B*, *C*, and *E*, respectively). During the postnatal interval P3 to adult, a large increase in PC1 immunoreactivity was observed in the nerve endings of the posterior lobe (Fig. 4 *C* and *E*).

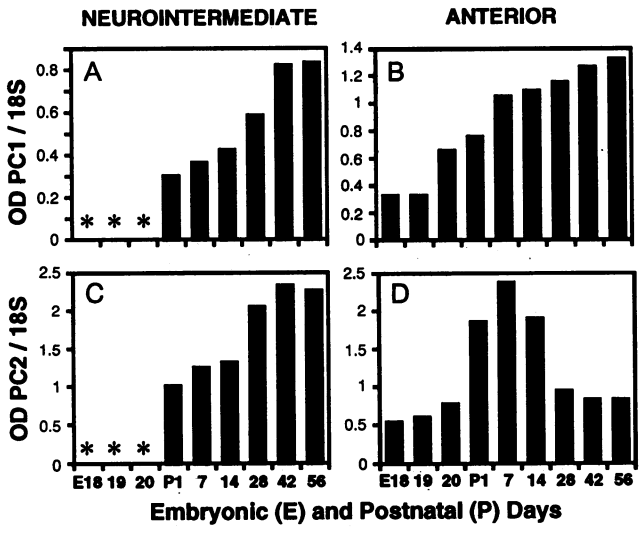


FIG. 3. Northern blot analysis of pituitary PC1 and PC2 during development. PC1 (*A* and *B*) and PC2 (*C* and *D*) hybridization of total RNA (10 μg) obtained from neurointermediate (*A* and *C*) and anterior (*B* and *D*) pituitary lobes at various developmental stages. The same blot was stripped by boiling and was reprobed for 18S rRNA. Relative quantitation of the integrated optical densities obtained from the autoradiograms was performed, normalizing each of the data points of the PC1 and PC2 2.8- to 3.0-kb bands (1) with the 18S rRNA band as described (9). Exposure times varied from 1 to several hours. *, not determined.

**PC1 and ACTH Colocalization.** Using the sequential immunostaining method of Tramu *et al.* (20), we demonstrated on the same histological section PC1-positive cells (Fig. 4*F*) and ACTH-containing cells (Fig. 4*G*). We clearly show that a majority of corticotrophs (Fig. 4*G*) were stained with the C-terminally directed PC1 antibody used (Fig. 4*F*), demonstrating the colocalization of ACTH and PC1 at the protein level. Other PC1-positive cells were observed and some of them have been characterized as gonadotrophs, since, in these cells, we showed the colocalization of PC1 (Fig. 4*H*) with βLH (Fig. 4*I*) and βFSH (data not shown).

**ICC Localization of PC2.** In the intermediate lobe, weak PC2 immunostaining was noted at E16 (Fig. 5*A*). This immunoreaction increased during the prenatal and postnatal periods and reached a plateau at adulthood (Fig. 4 *B–G*).

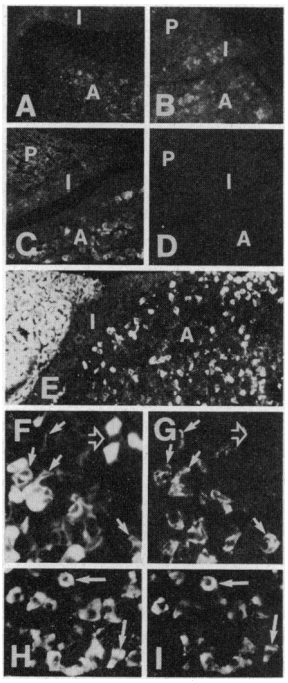


FIG. 4. PC1 ICC during development. PC1-specific immunofluorescence data are shown for E16 (*A*), P1 (*B*), P3 (*C*), and adulthood (*E*). Control obtained with the PC1 antigen preadsorbed antibodies is shown in *D*. By sequential immunostaining of the same section, pituitary PC1 (*F*) and ACTH (*G*) colocalization is shown at the cellular level (arrows). Some PC1-positive (*F*), but ACTH-negative (*G*) cells are indicated with open arrows. Colocalization of PC1 (*H*) and βLH (*I*) is also shown (solid arrows). Abbreviations are as in Fig. 1. (*A–D*, ×45; *E*, ×60; *F–I*, ×125.)

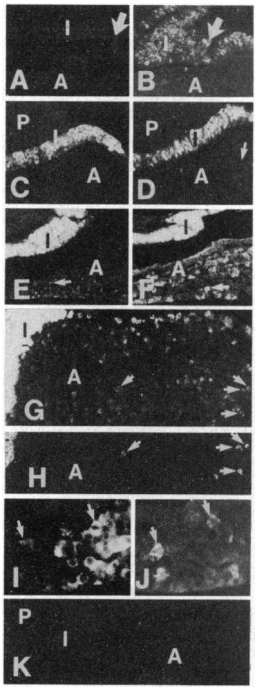


FIG. 5. PC2 ICC during development. PC2-specific immunofluorescence data are shown for E16 (*A*), E20 (*B*), P1 (*C*), P3 (*D*), P7 (*E*), P11 (*F*), and adulthood (*G*). Control immunostaining is shown in *K*. By sequential immunostaining of the same section, the pituitary PC2 (*G* and *I*) and αMSH (*H* and *J*) colocalization (arrows) are shown at adulthood (*G* and *H*) and at P7 (*I* and *J*). Abbreviations are as in Fig. 1. (*A–H*, ×40; *I* and *J*, ×80.)

In the anterior pituitary, we observed very little PC2 immunoreactivity on days E18 through P3 (Fig. 5 *A–D*). The PC2 immunoreactivity clearly appears starting on postnatal days P3–P14, is maximal at P11, and then decreases toward adulthood (Table 1; Fig. 5 *E–G*). These results generally agree with ISH data (Table 1; Fig. 2 *E–H*), also showing a transient increase in PC2 mRNA levels during the early postnatal period (see Table 1).

**Colocalization of αMSH and PC2 in the Adenohypophysis.** αMSH is produced mostly in the differentiating corticotrophs (13–16) and in a small number of atypical adult corticotrophs (11, 12). We therefore examined the possible cellular cohabitation of PC2 with αMSH in the early postnatal and adult anterior lobe. The colocalization of PC2 and αMSH in the adult anterior lobe is shown in Fig. 5 *G* and *H* and at postnatal day P7 in Fig. 5 *I* and *J*. Our data indicate that in the anterior lobe, αMSH-producing cells also contain PC2. Furthermore, the transient increase in αMSH immunoreactivity in the anterior lobe correlates with the transient increase in PC2 immunoreactivity (Fig. 5 *E* and *F*) and mRNA levels (Fig. 2 *F* and *G*). Although a good αMSH immunoreactivity was observed on E18 in the anterior lobe, the low levels of PC2 did not allow us to carry out unambiguous colocalization studies at this time point. Although not shown, PC2 colocalized also with βLH.

## DISCUSSION

In the present study, we describe the spatial and temporal expression of the proprotein and prohormone converting enzymes PC1 and PC2 during fetal development, postnatally, and in the adult mouse pituitary. During ontogeny, the expression of PC1 and PC2 mRNA levels shows tissue-specific variations. Our data correlate mRNA expression patterns of PC1 and PC2 (obtained by Northern blots and/or by ISH) with their ICC localization. PC1 and PC2 mRNA transcripts were first observed during late embryogenesis in a subset of endocrine cells of the developing mouse anterior pituitary. Analysis of the POMC cleavage specificity of the convertases (6, 7) and the present comparison of their expression to that of ACTH and αMSH demonstrates the involvement of PC1 and PC2 in the mechanism of cell-specific differential processing of POMC in the pituitary.

As summarized in Table 1, PC1 and PC2 mRNAs were first observed in the presumptive anterior lobe at E15, while intermediate lobe expression was detected only 1 day later. It was noted that PC1 and PC2 mRNAs were detected 1–2 days preceding the reliable detection of either protein by ICC. This delay is most likely due to the time necessary for accumulation of enough immunoreactive material. After the initial detection of PC1 and PC2 mRNA and protein in the anterior and intermediate lobes, their levels increased into adulthood. It is important to note that PC2 in the anterior lobe reached maximal levels during the first 2 weeks postnatally and then decreased to lower values in the adult anterior lobe. This transient increase in PC2 was demonstrated by ISH (Fig. 2 *E–H*), Northern blot analysis (Fig. 3*D*), and ICC (Fig. 5 *D–G*).

The ontogeny of PC1 and PC2 in the pituitary is comparable to that of the previously well studied POMC expression in mouse (16) and rat (15, 21). Similar to PC1 and PC2, anterior lobe POMC mRNA expression precedes that in the intermediate lobe by 1 day. An interval between the detection of POMC mRNA and protein is also observed in pituitary ontogeny. Interestingly, the anterior lobe POMC expression begins at E12.5 in mouse (16) and at E13 in rat (15, 21), preceding by almost 2 days that of PC1 and PC2 (which begins at E15). This may indicate that POMC is not cleaved early after its expression, and thus POMC processing would most likely begin after sufficient accumulation of the PC1 and PC2 enzymes. In support of this hypothesis, poor POMC processing has been described on E14 in the pituitary primordium (15, 22). Furthermore, >50% of POMC precursor remains unprocessed within the period E13.5–E17 in rat (23). In contrast, POMC cleavage is more efficient by E18 (22), a time when PC1 and PC2 are expressed at higher levels (Table 1). The low degree of POMC processing during the period E13–E15 may be due to the presence of other processing enzymes, such as furin, which can partially cleave POMC into βLPH (24). In this regard, furin is expressed in the rat anterior pituitary before day E15 (25). Therefore, the early (days E13–E15), but inefficient, processing of POMC may be mediated by furin or furin-like enzymes, while further efficient processing into multiple end products (22, 26) requires expression of PC1 and PC2.

Different POMC end products are produced in the intermediate and anterior lobes—i.e., mature corticotrophs synthesize mainly ACTH and βLPH with lower levels of β-endorphin, while melanotrophs process POMC to αMSH and β-endorphin as final products (26, 27). Therefore, is it possible to correlate the expression levels of PC1 and PC2 in the anterior and intermediate lobes with the POMC end products present? Our data show that in the adult mouse pituitary both PC1 and PC2 are expressed in melanotrophs and corticotrophs. More specifically, adult corticotrophs expressed principally PC1 mRNA and very little if any PC2 mRNA (9). In the present study, we confirm this observation at the ICC level. Thus, in a majority of adult corticotrophs, as detected with an ACTH antibody, we demonstrate PC1 immunoreactivity but very little PC2 immunoreactivity. The exception is a very small number of corticotrophs that are atypical and contain PC2 immunoreactivity. Only in these atypical corticotrophs do we also observe an αMSH-like immunoreactivity, most likely to be desacetyl-ACTH-(1–13) amide (27). The cleavage of ACTH-(1–39) at the Gly-Lys-Lys-Arg ↓ Arg-Pro site requires the specific action of PC2 (6) for production of the αMSH intermediate, ACTH-(1–17), and ACTH-(18–39). Therefore, it is not surprising that when PC2 mRNA expression or immunoreactivity is observed, αMSH-like material is also detected in POMC-expressing cells. The implication of PC2 in the processing of αMSH is further corroborated by the appearance of this enzyme within immature corticotrophs during the postnatal period P1–P14. POMC processing is well

characterized in this postnatal period (13–15, 28, 29). During this time interval, PC2 mRNA (Figs. 2 *F–H* and 3*D*) and protein levels (Fig. 5 *D–G*) transiently increase to reach their highest levels of expression during development. Also, at this time, the anterior lobe αMSH-like immunoreactivity, which is detected in all differentiating corticotrophs from the earliest stages (23) and persists during the first postnatal weeks, is at its highest level (13, 14, 28). The colocalization of PC2 and αMSH-like immunoreactivity in immature (and atypical) corticotrophs, presented in this work, supports the notion that a transient PC2 expression can be related to the transient production of αMSH-like immunoreactivity in the anterior lobe. Thus, variations in PC2 levels during ontogeny result in differential processing of POMC in the pituitary. The ratio of PC1/PC2 levels in POMC-expressing cells could be a critical point of control in the developmental plasticity of processing, including that of β-endorphin that usually follows the pattern of αMSH. Regardless, we cannot exclude the possibility that other proteases may intervene in ACTH cleavage (30).

The functional role of the αMSH-like peptide [desacetyl-ACTH-(1–13)-amide] and ACTH-(1–17) and ACTH-(18–39) produced in anterior pituitary lobe during early postnatal development is not clear. It has been proposed that αMSH has stimulatory effects on intrauterine growth, thus suggesting a trophic role for this peptide (31). It is possible that αMSH-like peptides may also have trophic actions during the early postnatal period when they are transiently produced in higher levels in the anterior pituitary. In this regard, αMSH has been shown to have growth-stimulating effects on the adrenal zona glomerulosa (32). Whatever the biological importance of these POMC-derived peptides, it is clear that their production is dependent on a transiently increased expression of PC2. This observation leads to the question of the mechanism responsible for the subsequent decrease in PC2 expression levels into adulthood. The specific factor(s) involved in the transient changes in anterior lobe PC2 expression has yet to be identified.

Another result of the present study that deserves mention is our partial analysis of the many anterior pituitary PC1-positive cells that are ACTH negative. Indeed, a majority of endocrine cells in this tissue are labeled for PC1, while corticotrophs represent only 10–15% of the cells. A large population of these ACTH-negative cells, which are intensely stained for PC1, appear to be gonadotrophs, as suggested by their position and shape. This observation was confirmed by the colocalization of PC1 with βLH and βFSH. Similarly, PC2 colocalized with βLH. The role of PC1 and PC2 in gonadotrophs awaits further studies addressing the question of the potential endogenous substrate(s) in these cells.

We also observed the immunoreactivity to PC1 (Fig. 4*E*) and PC2 (data not shown) in the posterior pituitary. Since there are no hybridizations within this lobe, the intense PC1 and weak PC2 immunoreactions can be attributed not to the pituicytes but to the nerve terminals. This suggests the production and transport of PC1 and PC2 from hypothalamic centers to the neurohypophysis and is in agreement with our earlier (1, 2) and recently more extensive (33) hypothalamic ISH data showing the high abundance of PC1 and PC2 mRNA in the supraoptic and paraventricular nuclei. Within the hypothalamic–neurohypophyseal system, one of the plausible roles of PC1 and PC2 is likely to be processing of the oxytocin and vasopressin precursors.

In conclusion, this work demonstrates the localization and ontogeny of the convertases PC1 and PC2 in pituitary. The histochemical evidence presented together with the deduced cleavage specificity (6) rationalizes the tissue and temporal changes in the processing of pituitary POMC. Finally, these results and all other available data suggest that PC1 and PC2 play a more general role in the control of developmental plasticity of precursor maturation.

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1. Seidah, N. G., Gaspar, L., Mion, P., Marcinkiewicz, M., Mbikay, M. & Chrétien, M. (1990) *DNA* **9**, 415–424.
2. Seidah, N. G., Marcinkiewicz, M., Benjannet, S., Gaspar, L., Beaubien, G., Mattei, M. G., Lazure, C., Mbikay, M. & Chrétien, M. (1991) *Mol. Endocrinol.* **5**, 111–122.
3. Smeekens, S. P., Avruch, A. S., LaMendola, J., Chan, S. J. & Steiner, D. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 340–344.
4. Smeekens, S. P. & Steiner, D. F. (1990) *J. Biol. Chem.* **265**, 2997–3000.
5. Seidah, N. G. & Chrétien, M. (1992) *Trends Endocrinol. Metabol.* **3**, 133–140.
6. Benjannet, S., Rondeau, N., Day, R., Chrétien, M. & Seidah, N. G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3564–3568.
7. Thomas, L., Leduc, R., Thorne, B. A., Smeekens, S. P., Steiner, D. F. & Thomas, G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5297–5301.
8. Benjannet, S., Reudelhuber, T., Mercure, C., Rondeau, N., Chrétien, M. & Seidah, N. G. (1992) *J. Biol. Chem.* **267**, 11417–11423.
9. Day, R., Schäfer, M. K.-H., Watson, S. J., Chrétien, M. & Seidah, N. G. (1992) *Mol. Endocrinol.* **6**, 485–497.
10. Seidah, N. G., Fournier, H., Boileau, G., Benjannet, S., Rondeau, N. & Chrétien, M. (1992) *FEBS Lett.* **310**, 235–239.
11. Girod, C. & Dubois, M. P. (1974) *C.R. Soc. Biol.* **168**, 751–754.
12. Tanaka, S. & Kurosumi, K. (1986) *Cell Tissue Res.* **243**, 229–238.
13. Khachaturian, H., Alessi, N. E., Munfakh, N. & Watson, S. J. (1983) *Life Sci.* **33**, Suppl. 1, 61–64.
14. Sato, S. M. & Mains, R. E. (1987) *Ann. N.Y. Acad. Sci.* **312**, 286–299.
15. Lugo, D. I., Roberts, J. L. & Pintar, J. E. (1989) *Mol. Endocrinol.* **3**, 1313–1324.
16. Hindelang, C., Félix, J. M., Laurent, F. M., Klein, M. J. & Stoeckel, M. E. (1990) *Mol. Cell. Endocrinol.* **70**, 225–235.
17. Marcinkiewicz, M., Benjannet, S., Seidah, N. G., Cantin, M. & Chrétien, M. (1987) *Cell Tissue Res.* **250**, 205–214.
18. Seidah, N. G., Day, R., Benjannet, S., Rondeau, N., Boudreault, A., Reudelhuber, T., Schäfer, M. K.-H., Watson, S. J. & Chrétien, M. (1992) *NIDA Res. Monogr.* **126**, 132–150.
19. Akil, H., Ueda, Y., Lin, H.-S. & Watson, S. J. (1981) *Neuropeptides* **1**, 429–446.
20. Tramu, G., Pillez, A. & Leonardelli, J. (1978) *J. Histochem. Cytochem.* **26**, 322–324.
21. Elkabes, S., Loh, Y. P., Nieburgs, A. & Wray, S. (1989) *Dev. Brain Res.* **46**, 85–95.
22. Allen, R. G., Pintar, J. E., Stack, J. & Kendall, J. W. (1984) *Dev. Biol.* **102**, 43–50.
23. Pintar, L. E. & Lugo, D. I. (1987) *Ann. N.Y. Acad. Sci.* **512**, 218–327.
24. Leduc, R., Thorne, B., Thomas, L., Smeekens, S., Steiner, D. & Thomas, G. (1990) *J. Cell Biol.* **111**, 338 (abstr.).
25. Zheng, M., Scott, R. E. M., Wood, T. L., Seidah, N. G. & Pintar, J. E. (1992) *Soc. Neurosci. Abstr.* **18**, 122.5.
26. Chrétien, M., Sikström, R. A., Lazure, C., Mbikay, M. & Seidah, N. G. (1989) *Biosci. Rep.* **9**, 693–700.
27. Mains, R. E. & Eipper, B. A. (1990) *Trends Endocrinol. Metabol.* **1**, 388–394.
28. Sato, S. M. & Mains, R. E. (1988) *Endocrinology* **122**, 68–77.
29. Noel, G. & Mains, R. E. (1991) *Endocrinology* **129**, 1317–1325.
30. Estivariz, F. E., Friedman, T. C., Chikuma, T. & Loh, Y. P. (1992) *J. Biol. Chem.* **267**, 7456–7463.
31. Swaab, D. F. & Boer, G. J. (1978) *Postgrad. Med. J.* **5**, Suppl. 1, 63–73.
32. Robba, C., Rebuffat, P., Mazzocchi, G. & Nussdorfer, G. G. (1986) *Acta Endocrinol.* **112**, 404–408.
33. Schäfer, M. K.-H., Day, R., Cullinan, W. E., Chrétien, M., Seidah, N. G. & Watson, S. J. (1993) *J. Neurosci.* **13**, 1258–1279.